OPINION

Nonalcoholic Fatty Liver Disease Progresses into Severe NASH when Physiological Mechanisms of Tissue Homeostasis Collapse

Silvia Sookoian,*,** Carlos J. Pirola*,***

* University of Buenos Aires, Institute of Medical Research A Lanari, Buenos Aires, Argentina.
** National Scientific and Technical Research Council (CONICET) - University of Buenos Aires, Institute of Medical Research (IDIM), Department of Clinical and Molecular Hepatology, Buenos Aires, Argentina.
*** National Scientific and Technical Research Council (CONICET)-University of Buenos Aires, Institute of Medical Research (IDIM), Department of Molecular Genetics and Biology of Complex Diseases, Buenos Aires, Argentina.

ABSTRACT

Phenotypic modulation of NAFLD-severity by molecules derived from white (adipokines) and brown (batokines) adipose tissue may be important in inducing or protecting against the progression of the disease. Adipose tissue-derived factors can promote the progression of NAFLD towards severe histological stages (NASH-fibrosis and NASHcirrhosis).
This effect can be modulated by the release of adipokines or batokines that directly trigger an inflammatory response in the liver tissue or indirectly modulate related phenotypes, such as insulin resistance. Metabolically dysfunctional adipose tissue, which is often infiltrated by macrophages and crown-like histological structures, may also show impaired production of anti-inflammatory cytokines, which may favor NAFLD progression into aggressive phenotypes by preventing its protective effects on the liver tissue.

Key words. NAFLD. NASH. Fibrosis. Metabolic stress.

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Hepatic neuregulin 4 signaling defines an endocrine checkpoint for steatosis-to-NASH progression.

Nonalcoholic fatty liver disease (NAFLD) is regarded as the most prevalent chronic liver disease worldwide.[1] Although the disease is considered benign and having a relatively good prognosis when the diagnosis is made at earlier histological stages, the progression into severe clinical forms, including nonalcoholic steatohepatitis (NASH), NASH-fibrosis and NASH-cirrhosis, imposes tremendous medical challenges.[1] For these reasons, extensive body of research aimed at the search for factors and or causes that modify the natural history of NAFLD is currently being conducted.

The complexity of the NAFLD-associated clinical picture, specifically the association with co-morbidities such as type 2 diabetes (T2D),[1] obesity and cardiovascular disease,[2-4] suggests the presence of an even more intricate network of pathogenic mechanisms that not only interact with each other but also increase the chances of having a more dramatic and severe phenotype. In this regard, the cross-talk between NAFLD and adipose tissue has gained particular attention of researchers and practitioners because cytokines derived from either white fat-WAT (adipokines) or brown fat-BAT (batokines) play a remarkable role in the development and maintenance of NAFLD disease severity. Figure 1 illustrates the concept of phenotypic modulation of NAFLD severity by molecules derived from adipose tissue that act as paracrine and endocrine hormones.[5,6]

Pathways involved in organ damage and injury, including cellular apoptosis or other process associated with cell death, as well as persistent tissue inflammation and the consequent fibrogenesis, are all triggered by multiple factors, including genetic predisposition and epigenetic modifiers.[7-9] Nevertheless, the exact mechanism/s that
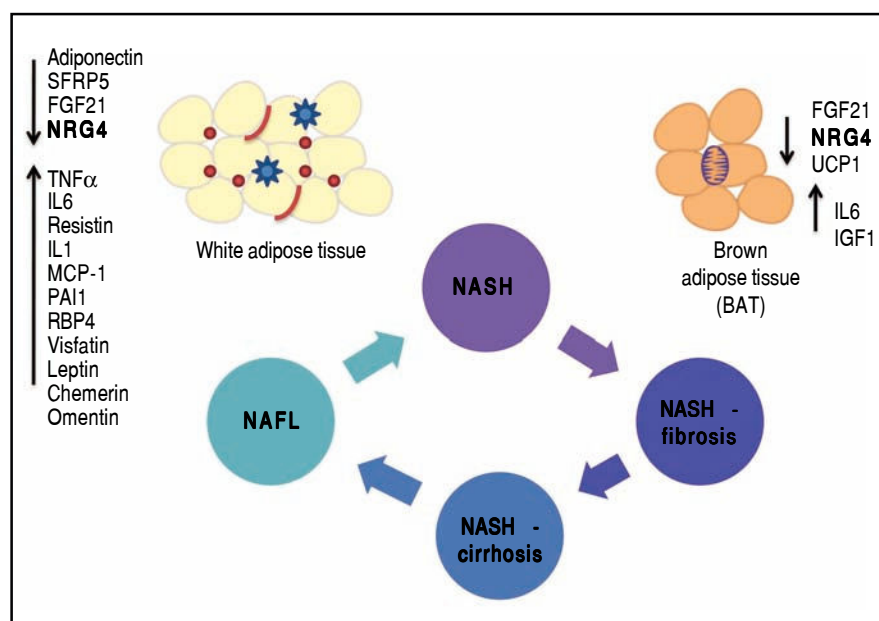
Figure 1. *Phenotypic modulation of NAFLD-severity by molecules derived from white (adipokines) and brown (batokines) adipose tissue by inducing or protecting against the progression of the disease. Adipose tissue-derived factors can promote the progression of NAFLD towards severe histological stages (NASH-fibrosis and NASH-cirrhosis). This effect can be modulated by the release of adipokines that directly trigger an inflammatory response in the liver tissue or indirectly modulate related phenotypes, such as insulin resistance (up-arrows). Metabolically dysfunctional adipose tissue, which is often infiltrated by macrophages and crown-like histological structures, may also show impaired production of anti-inflammatory adipokines, which may favor NAFLD progression into aggressive phenotypes by preventing its protective effects on the liver tissue (down-arrows). SFRP5: secreted frizzled-related protein 5. UCP1: uncoupling protein 1. FGF21: fibroblast growth factor 21. TNFα: tumor necrosis factor α. IL1 and IL6: interleukin 1 and 6. MCP-1: monocyte chemotactic protien-1.*

promote the transition of NAFL (nonalcoholic fatty liver) to NASH remain(s) elusive.

Guo, *et al.* recently reported the role of Neuregulin 4 *(Nrg4)*, an adipose tissue-enriched endocrine factor, in the development of NASH.[10] Specifically, the authors used a mouse model of high-fat-fructose diet-induced NASH that recapitulates main histological features of human NASH.[10] Key molecular findings associated with the presence of NASH in mice were:

- Cell death, which was associated with both increased phosphorylation of the member of the MAP-kinase family JNK1/2 and reduced protein levels of the inhibitor of apoptotic and necroptotic cell death c-FLIPL (Caspase 8 and FADD-Like Apoptosis Regulatory Protein, CFLAR), and
- Significantly decreased mRNA expression of *Nrg4* in epididymal WAT and BAT.[10]

Subsequent *Nrg4* gain- and loss-of-function studies in mouse models showed that restoration of *Nrg4* signaling protects animals from NASH; specifically, *Nrg4* signaling protects animals from stress-induced cell death through interaction with c-FLIP.[10] Notably, genes associated with fibrogenesis *(Col1a1, Acta2, Tgfb1, and Mmp13)* and inflammation *(Tnfa, Il1b, Il12b, Nos2, Ccl2, Ccl5, and Adgre1)* were upregulated in mice lacking *Nrg4*, suggesting that *Nrg4* deficiency exacerbated liver inflammation and fibrosis following NASH-diet feeding.[10] It is worth noting that a similar profile in human NAFLD was described by our research group.[2]

Considering the aforementioned findings collectively, it is plausible to hypothesize that NAFLD progression is a process in which physiological mechanisms of tissue homeostasis collapse. There is compelling evidence from human studies supporting this hypothesis. For instance, ballooning degeneration —a hallmark histological feature of NAFLD severity and NASH progression— is associated with down-regulation of liver HSP27 gene and protein expression.[11] HSP27 is a member of the heat shock family of proteins also known as stress-responsive protein 27. Furthermore, NASH development is associated with mitochondrial dysfunction, increased mitochondrial DNA (mtDNA) genetic diversity, down-regulation of liver expression of genes of the oxidative phosphorylation (OXPHOS) chain, and aberrant patterns of mtDNA methylation.[7,8,12,13]

The first question that immediately emerges is what factor/s trigger this complex picture?

Robust evidence supports the notion that NAFLD severity is associated with metabolic stress. In fact, it has been linked with increased transamination reactions that would promote coping with the liver metabolic derangement.[14] This, in turn, leads to changes in the amounts of amino acids released into the circulation, particularly in pathways related to glutamic acid.[13,14] As reported recently, glutaminolysis controls accumulation of myofibroblast hepatic stellate cells.[15] Deregulated liver metabolism also leads to aberrant patters of hedgehog signaling[16,17] and insufficient ketogenesis, which result in extensive hepatocyte injury and inflammation, decreased glycemia, and deranged hepatic TCA

(tricarboxylic acid) cycle intermediate concentrations.[14,18]

The second, and even more difficult to answer question is why a brown fat-derived secreted factor is important in maintaining liver tissue homeostasis?

It is well known that systemic metabolic homeostasis is regulated by the "in concert" action of a myriad of tissue-derived factors, which originate from different sources, including adipose tissue, gut, muscle, liver and bone. These hormones / growth factors / bioactive molecules basically sense global metabolism and energy homeostasis. These mechanisms may eventually fail to ensure cell / tissue physiological functions. Alternatively, they can simply malfunction because the affected cell / tissue has abnormally increased its metabolic demands, which might occur in common diseases like NAFLD and obesity, as well as in cancer. In the latter case, epidermal growth factors (EGFs) are critically implicated in cancer growth and survival by engaging a number of proteins via the ErbB (Tyrosine Kinase-Type Cell Surface Receptor HER2) family of proteins. In this regard, the neuregulins, including NRG4, activate type-1 growth factor receptors involved in initiating cell-to-cell signaling through tyrosine phosphorylation.[19] Neuregulins transduce signals via activation of the ErbB receptors, specifically ErbB4.

Evidence supporting the role of *Nrg4* in NAFLD and metabolic syndrome, while presently limited, is promising. For example, it has been shown that *Nrg4* attenuates hepatic lipogenic signaling and preserves glucose and lipid homeostasis in experimental models of obesity.[20] In this particular study, Wang, *et al.* demonstrated in mice that *Nrg4* negatively regulates *de novo* lipogenesis mediated by Lxr (liver X receptor) and Srebp1c (sterol regulatory element binding transcription factor 1) in a cell-autonomous manner.[20]

Data yielded by human studies is not only insufficient, but is also inconclusive. Kang, *et al.* found that the circulating NRG4 levels are increased in patients with type 2 diabetes and positively correlate with serum glucose level, HOMA-IR, and serum triglycerides;[21] however, opposite results were reported by Yan, *et al.*[22] Controversial evidence has also been derived from studies of gestational diabetes.[23,24] For example, Cai, *et al.* reported that NRG4 might protect against metabolic syndrome development.[25] Similarly, Dai, *et al.* found that patients with NAFLD also show reduced levels of serum NRG4.[26]

The question that still remains to be answered is whether the role of *Nrg4* in protecting hepatocytes from apoptosis and necroptosis triggered by metabolic stress is mediated by a circulating endocrine signaling released from WAT or BAT, or if it is a truly liver-specific derived *Nrg4*. NRG4 human liver gene and protein expression levels are considerably low (http://www.proteinatlas.org/ENSG00000169752-NRG4/tissue). Moreover, it is presently unclear whether *Nrg4* signaling is mediated by interaction with hepatocytes or whether the actual effector/s of *Nrg4* are non-liver related resident cells found in NAFLD and NASH. Hence, what exactly determines that *Nrg4* is a key molecule in protecting the liver from severe NAFLD is uncertain. There are many possibilities, including the fact that *Nrg4* indeed controls insulin sensitivity and secretion, as *Nrg4* seems to be a potent insulin releaser.[27] or macrophages migration into the liver. Still, Guo, *et al.* took the first steps toward unraveling the mechanisms involving the role of *Nrg4* in protecting fatty liver from severe NASH. A list of Gene Ontology (GO) terms and biologi-

Table 1. Gene Ontology (GO) - Biological Process for NRG4 Gene.

GO ID	GO term.
GO:0000165	MAPK cascade.
GO:0014066	Regulation of phosphatidylinositol 3-kinase signaling.
GO:0018108	Peptidyl-tyrosine phosphorylation.
GO:0035556	Intracellular signal transduction.
GO:0038111	Interleukin-7-mediated signaling pathway.
GO:0038128	ERBB2 signaling pathway.
GO:0043547	Positive regulation of GTPase activity.
GO:0046854	Phosphatidylinositol phosphorylation.
GO:0048015	Phosphatidylinositol-mediated signaling.
GO:0048513	Animal organ development.
GO:1901185	Negative regulation of ERBB signaling pathway.
GO:2000145	Regulation of cell motility.

NRG4 (Neuregulin 4) is a protein coding gene. Among its related pathways are RET signaling (REarranged during Transfection) and signaling by ERBB2. RET (Ret Proto-Oncogene) encodes one of the receptor tyrosine kinases, which are cell-surface molecules that transduce signals for cell growth and differentiation. ERBB2 (Erb-B2 Receptor Tyrosine Kinase 2) encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases; this protein has no ligand binding domain of its own and therefore cannot bind growth factors. GO annotations related to NRG4 include receptor binding and growth factor activity.
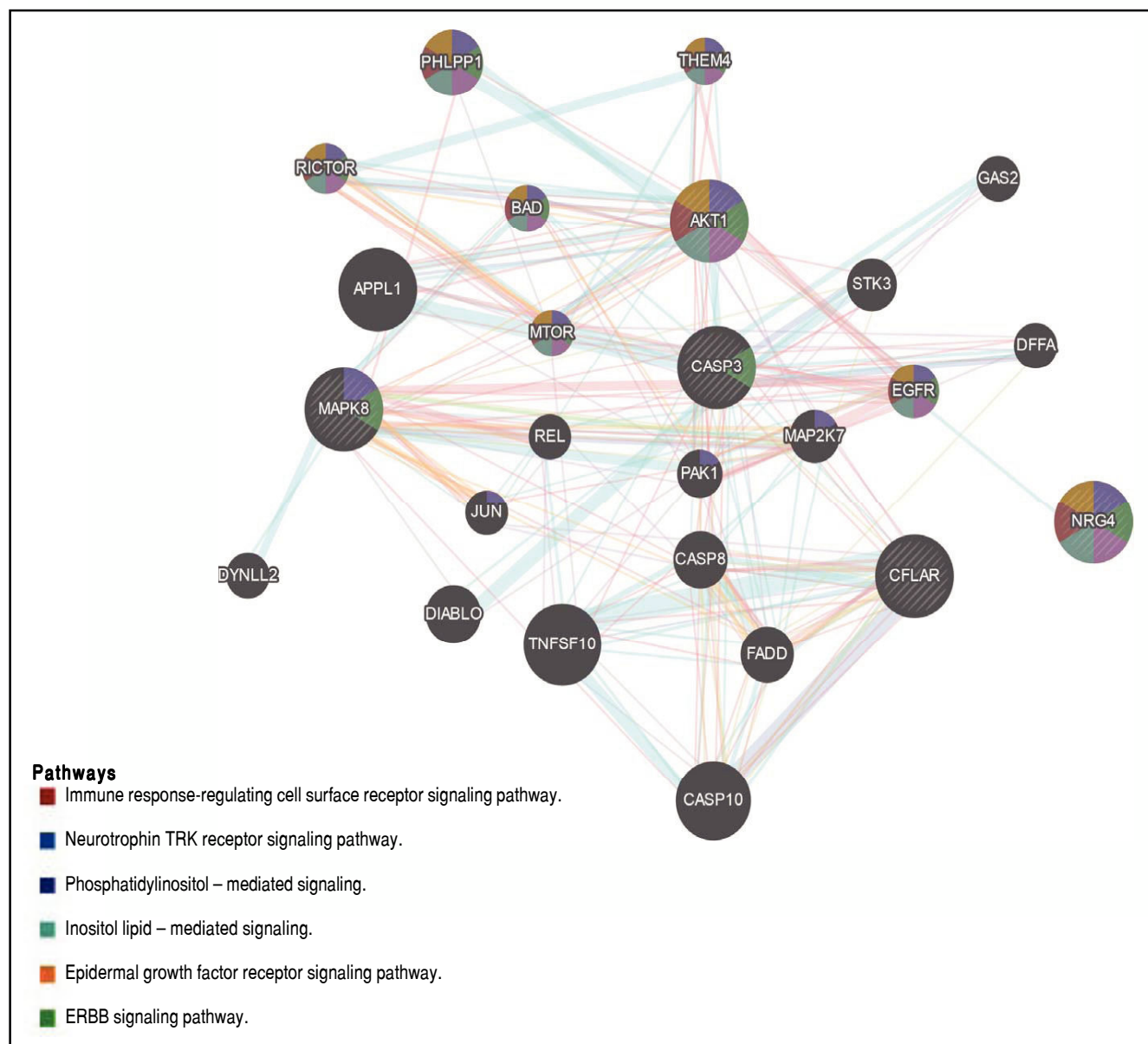
Figure 2. *NRG4 interaction network. Gene-gene functional network construction was done using GeneMANIA, available at: https://github.com/GeneMANIA/genemania; visualization was performed by Cytoscape (http://apps.cytoscape.org/apps/GeneMania). NRG4: neuregulin 4; CFLAR CASP8 and FADD like apoptosis regulator; MAPK8: mitogen-activated protein kinase 8. CASP3: caspase 3. AKT1: AKT serine/threonine kinase 1. APPL1: adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1. TNFSF10: tumor necrosis factor superfamily member 10. CASP10: caspase 10. PHLPP1: PH domain and leucine rich repeat protein phosphatase 1. DIABLO: diablo IAP-binding mitochondrial protein. CASP8: caspase 8. FADD: Fas associated via death domain. EGFR: epidermal growth factor receptor. STK3: serine/threonine kinase 3. MAP2K7: mitogen-activated protein kinase kinase 7. RICTOR: RPTOR independent companion of MTOR complex 2. PAK1: p21 (RAC1) activated kinase 1. BAD: BCL2 associated agonist of cell death. THEM4: thioesterase superfamily member 4. MTOR: mechanistic target of rapamycin. DFFA: DNA fragmentation factor subunit alpha. DYNLL2: dynein light chain LC8-type 2. GAS2: growth arrest specific 2. JUN: Jun proto-oncogene. AP-1 transcription factor subunit. REL REL proto-oncogene. NF-κB subunit.*

cal process for NRG4 gene and its interaction network are shown in table 1 and figure 2, respectively. It is clear that this molecule seems to play a key role in ensuring effective protection against the progression of NAFLD by opposing caspase-mediated cell death. Interestingly, human "knock-out" for NRG4 has been identified; however, its phenotypic manifestation in the liver remains to be characterized.[28]

In conclusion, there is substantial evidence suggesting that dysregulation of molecular mediators of metabolic

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homeostasis modulates the natural history of NAFLD. Both their upregulation and deficiency can either cause or exacerbate an adverse phenotype by evading from protective mechanisms against liver damage (Figure 1). Hence, the evolution of NAFLD into severe NASH is mediated by loss of systemic and/ or local protective mechanisms of organ damage.

REFERENCES

1. Brunt EM, Wong VW, Nobili V, Day CP, Sookoian S, Maher JJ, Bugianesi E, et al. Nonalcoholic fatty liver disease. *Nat Rev Dis Primers* 2015; 17: 15080.
2. Sookoian S, Gianotti TF, Rosselli MS, Burgueno AL, Castano GO, Pirola CJ. Liver transcriptional profile of atherosclerosis-related genes in human nonalcoholic fatty liver disease. *Atherosclerosis* 2011; 218: 378-85.
3. Sookoian S, Castano GO, Burgueno AL, Rosselli MS, Gianotti TF, Mallardi P, Martino JS, et al. Circulating levels and hepatic expression of molecular mediators of atherosclerosis in non-alcoholic fatty liver disease. *Atherosclerosis* 2010; 209: 585-91.
4. Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: a systematic review. *J Hepatol* 2008; 49: 600-7.
5. Adolph TE, Grander C, Grabherr F, Tilg H. Adipokines and Non-Alcoholic Fatty Liver Disease: Multiple Interactions. *Int J Mol Sci* 2017 29; 18(8).
6. Polyzos SA, Kountouras J, Mantzoros CS. Adipokines in nonalcoholic fatty liver disease. *Metabolism* 2016; 65: 1062-79.
7. Sookoian S, Rosselli MS, Gemma C, Burgueno AL, Fernandez GT, Castano GO, Pirola CJ. Epigenetic regulation of insulin resistance in nonalcoholic fatty liver disease: impact of liver methylation of the peroxisome proliferator-activated receptor gamma coactivator 1alpha promoter. *Hepatology* 2010; 52: 1992-2000.
8. Pirola CJ, Scian R, Gianotti TF, Dopazo H, Rohr C, Martino JS, Castano GO, et al. Epigenetic Modifications in the Biology of Nonalcoholic Fatty Liver Disease: The Role of DNA Hydroxymethylation and TET Proteins. *Medicine (Baltimore)* 2015; 94: e1480.
9. Sookoian S, Pirola CJ. Genetic predisposition in nonalcoholic fatty liver disease. *Clin Mol Hepatol* 2017; 23: 1-12.
10. Guo L, Zhang P, Chen Z, Xia H, Li S, Zhang Y, Kobberup S, et al. Hepatic neuregulin 4 signaling defines an endocrine checkpoint for steatosis-to-NASH progression. *J Clin Invest* 2017 November 6 [Epub ahead of print].
11. Sookoian S, Castano GO, Scian R, San MJ, Pirola CJ. Heat Shock Protein 27 is down-regulated in Ballooned Hepatocytes of Patients with Nonalcoholic Steatohepatitis (NASH). *Sci Rep* 2016; 3:22528.
12. Sookoian S, Flichman D, Scian R, Rohr C, Dopazo H, Gianotti TF, Martino JS, et al. Mitochondrial genome architecture in non-alcoholic fatty liver disease. *J Pathol* 2016; 240: 437-49.
13. Sookoian S, Pirola CJ. The NASH Metabotype: Imbalance of Circulating Amino Acids and Transamination Reactions Reflect Impaired Mitochondrial Function. *Hepatology* 2017 [Epub ahead of print]
14. Sookoian S, Castano GO, Scian R, Fernandez GT, Dopazo H, Rohr C, Gaj G, et al. Serum aminotransferases in nonalcohol-ic fatty liver disease are a signature of liver metabolic perturbations at the amino acid and Krebs cycle level. *Am J Clin Nutr* 2016; 103: 422-34.
15. Du K, Hyun J, Premont RT, Choi SS, Michelotti GA, Swiderska-Syn M, Dalton GD, et al. Hedgehog-YAP Signaling Pathway Regulates Glutaminolysis to Control Hepatic Stellate Cell Activation. *Gastroenterology* 2018 [Epub ahead of print].
16. Chen Y, Choi SS, Michelotti GA, Chan IS, Swiderska-Syn M, Karaca GF, Xie G, et al. Hedgehog controls hepatic stellate cell fate by regulating metabolism. *Gastroenterology* 2012; 143: 1319-29.
17. Verdelho MM, Diehl AM. Role of Hedgehog Signaling Pathway in NASH. *Int J Mol Sci* 2016; 17(6).
18. Cotter DG, Ercal B, Huang X, Leid JM, d'Avignon DA, Graham MJ, Dietzen DJ, et al. Ketogenesis prevents diet-induced fatty liver injury and hyperglycemia. *J Clin Invest* 2014; 124: 5175-90.
19. Harari D, Tzahar E, Romano J, Shelly M, Pierce JH, Andrews GC, Yarden Y. Neuregulin-4: a novel growth factor that acts through the ErbB-4 receptor tyrosine kinase. *Oncogene* 1999; 18: 2681-9.
20. Wang GX, Zhao XY, Meng ZX, Kern M, Dietrich A, Chen Z, Cozacov Z, et al. The brown fat-enriched secreted factor *Nrg4* preserves metabolic homeostasis through attenuation of hepatic lipogenesis. *Nat Med* 2014; 20(12): 1436-43.
21. Kang YE, Kim JM, Choung S, Joung KH, Lee JH, Kim HJ, Ku BJ. Comparison of serum Neuregulin 4 (*Nrg4*) levels in adults with newly diagnosed type 2 diabetes mellitus and controls without diabetes. *Diabetes Res Clin Pract* 2016; 117: 1-3.
22. Yan PJ, Xu Y, Wan Q, Feng J, Li H, Gao CL, Yang J, et al. Decreased plasma neuregulin 4 concentration is associated with increased high-sensitivity C-reactive protein in newly diagnosed type 2 diabetes mellitus patients: a cross-sectional study. *Acta Diabetol* 2017; 54: 1091-9.
23. Kralisch S, Hoffmann A, Kratzsch J, Bluher M, Stumvoll M, Fasshauer M, Ebert T. The brown-fat-secreted adipokine neuregulin 4 is decreased in gestational diabetes mellitus. *Diabetes Metab* 2017 [Epub ahead of print].
24. Kurek EM, Yayla AC, Sahin EG, Altun ET, Pekin O, Cevik O. Clinical significance of neuregulin 4 (*NRG4*) in gestational diabetes mellitus. *Gynecol Endocrinol* 2017; 28: 1-4.
25. Cai C, Lin M, Xu Y, Li X, Yang S, Zhang H. Association of circulating neuregulin 4 with metabolic syndrome in obese adults: a cross-sectional study. *BMC Med* 2016; 14: 165.
26. Dai YN, Zhu JZ, Fang ZY, Zhao DJ, Wan XY, Zhu HT, Yu CH, et al. A case-control study: Association between serum neuregulin 4 level and non-alcoholic fatty liver disease. *Metabolism* 2015; 64: 1667-73.
27. South JC, Blackburn E, Brown IR, Gullick WJ. The neuregulin system of ligands and their receptors in rat islets of langerhans. *Endocrinology* 2013; 154: 2385-92.
28. Saleheen D, Natarajan P, Armean IM, Zhao W, Rasheed A, Khetarpal SA, Won HH, et al. Human knockouts and phenotypic analysis in a cohort with a high rate of consanguinity. *Nature* 2017; 544: 235-9.

Correspondence and reprint request:
Silvia Sookoian, M.D., Ph.D.
Instituto de Investigaciones Médicas, IDIM-CONICET
Combatientes de Malvinas 3150, CABA-1427, Argentina.
Tel.: 54-11-52873905
E-mail: sookoian.silvia@lanari.fmed.uba.ar